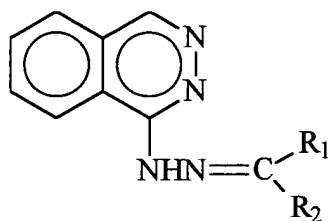


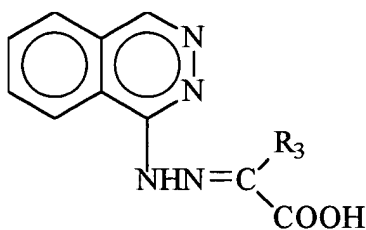
Stable Pharmaceutical Compositions

Background of the Invention

The present invention relates to both a novel method of stabilizing hydralazine hydrochloride in pharmaceutical preparations and pharmaceutical compositions containing stabilized hydralazine compounds having the general formula:



or compounds having the formula:



where R_1 and R_2 are independently H, branched or straight chain alkyl having from 1 to about 7 carbon atoms, substituted or unsubstituted aryl, substituted or unsubstituted

cycloalkyl, substituted or unsubstituted aralkyl, substituted or unsubstituted alkylcycloalkyl, lower alkenyl or R₁ and R₂ together form part of a substituted or unsubstituted cycloalkyl having from about 4 to about 7 carbon atoms; where R₃ is a branched or straight chain alkyl having from 1 to about 7 carbon atoms, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, substituted or unsubstituted cycloalkyl, aralkyl, substituted or unsubstituted alkylcycloalkyl or a group having the formula (CH₂)_nCOOH where n is from 1 to about 7.

Hydralazine hydrochloride is a peripheral vasodilator discovered about 50 years ago that exerts an antihypertensive effect directly on vascular smooth muscle producing relaxation of muscle fibers resulting in a decrease in blood pressure. Hydralazine is extensively metabolized in the body to products that are excreted predominantly in the urine, and undergoes N-acetylation, oxidation, hydroxylation, hydrazone formation and conjugation.

Commercially available in both oral and injectable dosage forms, hydralazine is used to lower blood pressure in hypertensive crisis situations and in patients requiring long-term management of their hypertension after the crisis has abated. Hypertensive crisis is a medical emergency that requires immediate therapy for certain patients in hospital emergency rooms, operating rooms and intensive care units.

Hydralazine is an artery specific direct peripheral vasodilator having an onset of action between 10-30 minutes (10-20 minutes given intravenously), a maximum hypotensive effect in 10-80 minutes and duration of action between 3-4 hours. Hydralazine is one of the few injectable antihypertensive drugs that maintain blood flow to kidneys during hypertensive crisis, and the only one to increase blood flow to an already compromised kidney. Although the drug is approved for administration of 20-40 mg doses, there are several clinical hazards associated with the currently available hydralazine formulation. First, the instability of the 20 mg/ml sterile solutions is a serious problem and has frequently caused removal of the product from the market by the FDA. Submicron

1 particles appear in the hydralazine sterile injection solutions during storage for more than
2 6-12 months. Secondly, the concentrated 20mg/ml dosage form of hydralazine is difficult
3 to administer accurately to patients at the small doses (3-5 mg) required to avoid
4 “overshoot” hypotension. Consequently, these concentrated solutions are generally
5 diluted prior to use in the hospital. Unfortunately, dilution by hospital personnel in an
6 attempt to reduce the administration problems risks alteration of the hydralazine product,
7 metal contamination and generation of toxic substances. Commercially available
8 hydralazine solutions discolor when inappropriately diluted with metal-containing or
9 carbohydrate-containing diluents generally found in hospitals. The Food and Drug
10 Administration (FDA)s labeling for the currently available hydralazine formulation
11 indicates that hydralazine should not be added to infusion solutions, and that hydralazine
12 hydrochloride injections may discolor upon contact with metal. The FDA further warns
13 in the product labeling that discolored solutions should be discarded.

14
15 Hypertensive crisis is a life-threatening situation and includes hypertensive emergencies
16 and hypertensive urgencies characterized by acute elevations in blood pressure, which
17 must be brought under control within hours. Over 60 million people in the United States
18 suffer from essential hypertension. About 1% of these people suffers from hypertensive
19 crisis and requires hospital-based acute care. Of the hypertensive crisis patients, 76 % are
20 “urgencies” and 24% “emergencies” with end-organ damage. Hypertensive urgencies are
21 those situations in which it is desirable to reduce blood pressure quickly; however,
22 hypertensive urgencies can be managed without requiring rapid, controlled reduction of
23 blood pressure. Elevated blood pressure alone, in the absence of symptoms or progressive
24 target organ damage rarely requires emergency treatment. Hypertensive urgencies are
25 treated with oral antihypertensives such as nifedipine or clonidine, or with intravenous
26 labetalol.

27
28 Hypertensive emergencies are characterized by acute elevations in blood pressure
29 (diastolic >110 to 120 mm Hg) which can potentially be life threatening and thus require
30 rapid, controlled reduction of blood pressure. Prompt pharmacologic therapy is indicated

1 for those patients having Stage 2 ($\geq 160/100$ mm Hg) or Stage 3 ($\geq 180/110$ mm Hg)
2 hypertension who have clinically manifested cardiovascular disease or target organ
3 damage. The most commonly used antihypertensive agent is nitroprusside. Although
4 hydralazine is already labeled for severe essential hypertension when oral hydralazine
5 cannot be given or when the need to lower blood pressure is urgent as in hypertensive
6 crisis, it is not currently labeled for hypertensive emergencies when a patient presents
7 with emergent end-organ damage. As a patient's blood pressure is acutely elevated, the
8 patient experiences a dramatic decrease in blood flow to vital tissues such as the kidney
9 and brain. The reduction in elevated blood pressure in these patients through
10 antihypertensive therapy is important because it minimizes ischemic damage resulting
11 from reduced blood flow to these tissues. Examples of emergent end-organ damage
12 include hypertensive encephalopathy, cerebral infarction, intracranial hemorrhage,
13 myocardial ischemia, acute pulmonary edema, hypertensive nephropathy, hypertensive
14 retinopathy and eclampsia. The goal of therapy in hypertensive emergencies is to reduce
15 the mean arterial pressure by no more than 25 percent with two hours, then toward
16 160/110 mm Hg within 2 to 6 hours avoiding excessive drops in pressure that may
17 precipitate or aggravate renal, cerebral or coronary ischemia. Ultimately, the goal of
18 therapy is to reduce the blood pressure to below 140/90 mm Hg.

19
20 Hydralazine hydrochloride is very unstable in all of the injectable pharmaceutical
21 formulations currently commercially available. Continuing instability problems with
22 injectable hydralazine hydrochloride, for example, have plagued pharmaceutical
23 manufacturers for many years, forcing these companies to remove their injectable
24 hydralazine products from the marketplace. Although a shelf life of 12 months is
25 currently required for FDA approval for injectable hydralazine hydrochloride, only a few
26 companies have been able to satisfy this requirement with adequate stability data. One
27 such company, SoloPak Pharmaceuticals, Inc. was able to meet the 12-month stability
28 requirements for FDA approval; however, the company was not able to provide a drug
29 product that was consistently stable for more than 6 months.

1 In its injectable formulation, hydralazine forms small yellow-green particles following
2 storage from 1 to about 2 months when hydralazine is stored at 40° C and after from 6 to
3 about 9 months storage at 25° C. Although the identification of the yellow-green particles
4 has yet to be confirmed, it is believed that the particles are insoluble polymeric products
5 formed during storage of hydralazine. It is believed that hydralazine hydrochloride
6 undergoes degradation in stored sterile injectable solutions to insoluble polymeric
7 products due to the highly reactive hydrazino group. Hydralazine hydrochloride also
8 undergoes several pharmaceutically undesirable reactions such as chelation with metal
9 ions, oxidation, and pH-dependent decomposition. It is believed that these reactions,
10 which often cause discoloration of hydralazine compositions, are also due to the highly
11 reactive hydrazino group.

12
13 Kanazawa et al. [Chemical and Pharmaceutical Bulletin 34(4):1840-1842 (1996)] report
14 that during the storage of a prescription admixture of pulverized hydralazine
15 hydrochloride with cimetidine, a histamine H₂-receptor antagonist for duodenal ulcer, the
16 initially uncolored admixture gradually turns to pale yellow. Kanazawa et al. further
17 report that hydralazine hydrochloride undergoes degradation and discoloration with
18 cimetidine in aqueous solution to give 1, 1-di (phthalazin-3-yl) amine, 1, 1-di (phthalazin-3-
19 yl) hydrazine, 1-amino-1, 2, 2-tri- (phthalazin-3-yl) hydrazine, and 1, 1, 2-tri (phthalazin-3-
20 yl) hydrazine.

21
22 Alexander et al. [American Journal of Hospital Pharmacy 50: 683- 686(1993)] report that
23 the degradation of hydralazine hydrochloride in a sugar-containing oral syrup was quite
24 fast and was apparently a first order process. The authors report that sugar (e.g., dextrose
25 and fructose) reduces the stability of hydralazine hydrochloride considerably. Their
26 syrup containing maltitol normally increases the stability of drugs sensitive to the
27 presence of sugars; however, the hydralazine formulation remained unstable at room
28 temperature.

1 Lessen et al. [Journal of Pharmaceutical sciences 85(3): 326- 329(1996)] report that the
2 strength of hydralazine hydrochloride in 10 mg tablets containing starch as an excipient
3 decreases significantly with time and produced fluorescence at 414 nm. Lessen et al.
4 report that, in addition to the usual hydralazine degradants such as phthalazone and
5 phthalazine, these tablet compositions produced triazolophthalazine derivatives.

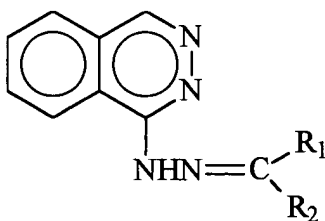
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7 Hydralazine is known to chelate metal ions. Sinha and Motten [Biochemical and
8 Biophysical Research Communications 105(3): 1044- 1051(1982)] report that
9 hydralazine oxidizes rapidly in the presence of oxygen and metal compounds such as
10 Cu^{+2} , Fe^{+2} , and Fe^{+3} through free radical intermediates much like other hydrazine
11 derivatives.

12
13 Because of its reactivity toward metals, standard manufacturing requirement for the
14 preparation of bulk hydralazine solution or sterile fill solution is that neither should come
15 into contact with any metal surface including tanks, transfer lines or filling lines.
16 Unfortunately, these precautions can be consistently enforced by the manufacturer only
17 during preparation and storage of the hydralazine solutions. After storage of the
18 hydralazine solutions, the handling of the hydralazine solutions is no longer under their
19 control.

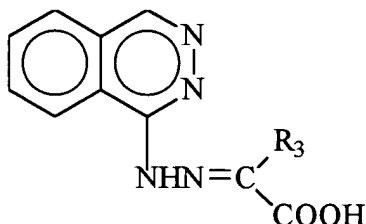
20 Despite its unique pharmacologic properties as a hypertensive drug, the therapeutic use of
21 hydralazine hydrochloride has been limited by its instability during storage and
22 difficulties in handling by medical personnel. A stable hydralazine pharmaceutical
23 composition that is more easily manufactured and does not degrade or produce particulate
24 matter during extended storage does not currently exist. Moreover, an injectable
25 hydralazine pharmaceutical formulation that is not adversely affected by conventional
26 dilution techniques in the hospital does not currently exist. This, despite the fact that
27 hydralazine was discovered as an antihypertensive agent over 50 years ago. A stable
28 hydralazine composition that could be manufactured more easily and stored for periods of
29 time greater than the current 12 month limit represents a significant advance.

Brief Summary of the Invention

This invention relates to a method of improving the stability of a hydralazine composition during manufacturing or storage comprising coupling an N-protecting group with hydralazine to produce the compound having the formula:



or a compound having the formula:



where R₁ and R₂ are independently H, branched or straight chain alkyl having from 1 to about 7 carbon atoms, substituted or unsubstituted aryl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted aralkyl, substituted or unsubstituted alkylcycloalkyl, lower alkenyl or R₁ and R₂ together form part of a substituted or unsubstituted cycloalkyl having from about 4 of about 7 carbon atoms; where R₃ is a branched or straight chain alkyl having from 1 to about 7 carbon atoms, substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, substituted or unsubstituted

1 cycloalkyl, aralkyl, substituted or unsubstituted alkylcycloalkyl or a group having the
2 formula $(CH_2)_nCOOH$ where n is from 1 to about 7; and
3 where said N-protecting group is removed from said compound after manufacturing or
4 storage.

5

6 It is an object of the present invention to stabilize pharmaceutical compositions during
7 manufacturing so that the hydralazine does not react with metal components of the
8 manufacturing system.

9

10 It is a further object of the present invention to extend the shelf life of oral and injectable
11 pharmaceutical compositions containing hydralazine significantly beyond 12 months
12 storage and preferably beyond 24 months storage.

13

14 It is a further object of the present invention to stabilize injectable pharmaceutical
15 compositions containing hydralazine during storage and reduce the formation of
16 submicron particles.

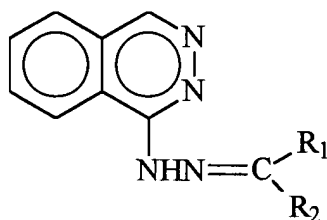
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18 It is a further object of the present invention to stabilize injectable pharmaceutical
19 compositions containing hydralazine and reduce the discoloration of the hydralazine
20 solution when diluted with conventional pharmaceutical diluents.

21

Detailed Description of the Invention

Compounds in accordance with the one embodiment of the present invention include those containing a non-toxic, biocompatible N-protecting group on the highly reactive hydrazine group of hydralazine represented by the formula:

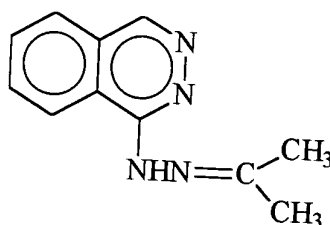


where R_1 and R_2 are independently H, substituted or unsubstituted branched or straight chain alkyl having from 1 to about 7 carbon atoms, substituted or unsubstituted aryl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted aralkyl, substituted or unsubstituted alkylcycloalkyl, lower alkenyl or R_1 and R_2 together form part of a substituted or unsubstituted cycloalkyl having from about 4 of about 7 carbon atoms.

In one aspect of the present invention, R_1 and R_2 are preferably unsubstituted branched or straight chain lower alkyls including but not limited to methyl, ethyl, propyl, isopropyl, butyl, isobutyl, pentyl, isopentyl and hexyl groups. In another aspect of the present invention, R_1 and R_2 are substituted with hydroxyls. In this embodiment, R_1 is H and R_2 has the formula $\text{CH}_2(\text{CHOH})_m\text{CH}_2\text{OH}$ where m is 2 or 3.

In one embodiment of the present invention, R_1 and R_2 are both branched or straight chain lower alkyls. In another embodiment of the present invention, R_1 is a substituted or unsubstituted aryl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted aralkyl, substituted or unsubstituted alkylcycloalkyl and R_2 is H or lower alkyl. In yet another embodiment of the present invention, R_1 and R_2 are both substituted or unsubstituted aryl, substituted or unsubstituted cycloalkyl, substituted or unsubstituted aralkyl, substituted or unsubstituted alkylcycloalkyl, lower alkenyl or R_1 and R_2 together

1 form part of a substituted or unsubstituted cycloalkyl having from about 4 of about 7
2 carbon atoms. In one preferred embodiment of the present invention, R₁ is methyl and R₂
3 hydrogen. In a more preferred embodiment of the present invention, compounds of the
4 present invention include acetone, 1-phthalazinyldiazine having the formula:
5



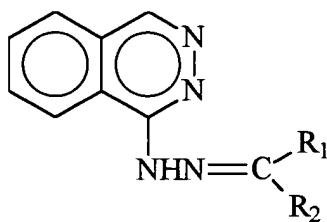
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7
8 The preparation of acetone, 1-phthalazinyldiazine is described in United States Patent
9 2,484,029 issued on October 11, 1949, which is hereby incorporated by reference;
10 however, no information regarding its stability in aqueous solution is provided.
11

12 Compounds in accordance with this embodiment of the present invention are readily
13 prepared by reaction of the carbonyl group of the desired acetone or aldehyde with the
14 highly reactive primary amino group of hydralazine (1-phthalazinyldiazine). The
15 resulting derivative of hydralazine is generally called a hydrazone. Although aldehydes
16 and ketones are widespread in nature and are generally non-toxic, in preferred
17 embodiments of the present invention these aldehydes and ketones will eventually end up
18 in the patient's plasma after being released from the hydralazine parent compound.
19 Preferably, therefor, the aldehydes and ketones are non-toxic and biocompatible, and do
20 not cause any deleterious effects in animals. Certain aldehydes and ketones have already
21 been recognized as less toxic and of lower risk to human health by the FDA and are
22 referred to as Class 3 compounds. These so-called Class 3 compounds include those not
23 known as a human health hazard at levels normally accepted in pharmaceuticals even
24 though there are no long-term toxicity or carcinogenicity studies for many of the
25 compounds. Available data indicate that they are less toxic in acute or short-term studies

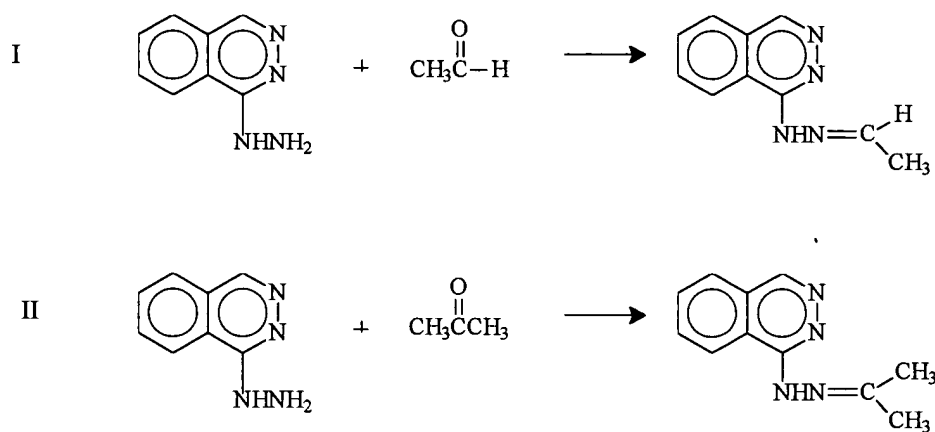
1 and negative in genotoxicity studies. It is considered that small amounts of these
2 compounds in the amount of 50 mg per day or less (corresponding to 5,000 ppm would
3 be acceptable without justification.

4
5 These hydrazone forming reactions are generally catalyzed by a small amount of acid,
6 and are buffered to a pH of about 4 to 5. In agreement with general acid catalysis in
7 which the conjugate acid of the carbonyl reactant combines with a free amino group, the
8 rate at which these compounds are formed generally drops at higher and lower pH values.
9 At high pH there will be a vanishingly low concentration of the carbonyl conjugate acid,
10 and at low pH most of the amine reactant will be tied up as its ammonium conjugate acid.
11 In general, these types of derivatization reactions do not require active removal of water,
12 and the products often precipitate from solution as they are formed.

13
14 In one embodiment of the present invention, hydralazine derivatives are prepared by
15 coupling an N-protecting group to the terminal nitrogen of the highly reactive hydrazino
16 group on hydralazine to produce a compound having the formula:



17
18
19
20 where R₁ and R₂ are independently H, lower alkyl, or lower alkenyl. Because the
21 hydrazine group readily reacts with the carbonyl group of acetone and aldehydes the N-
22 protected compounds of the present invention can easily be prepared by reacting
23 hydralazine hydrochloride with aldehydes such as formaldehyde and acetaldehyde (I) or
24 ketones such as acetone and other lower alkyl ketones such as butanone (II) as illustrated
25 generally below:






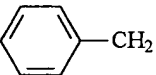
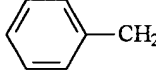
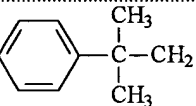
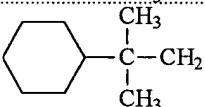
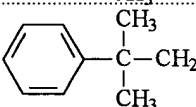
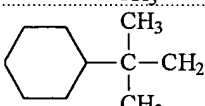
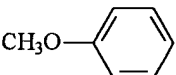


Illustrative compounds in accordance with this embodiment of the present invention are found in Table 1.

1

Table 1 - Ketones and Aldehydes

Compound Number	R ₁	R ₂	Starting Material
1	H	H	formaldehyde
2	CH ₃	H	acetaldehyde
3	CH ₃	CH ₃	acetone
4	CH ₂ CH ₃	H	propionaldehyde
5	CH ₂ CH ₃	CH ₃	2-butanone
6	CH ₂ CH ₃	CH ₂ CH ₃	3-pentanone
7	CH ₂ CH ₃	CH ₂ CH ₂ CH ₃	3-hexanone
8	CH ₂ CH ₂ CH ₃	H	1-butyraldehyde
9	CH ₂ CH ₂ CH ₃	CH ₃	2-pentanone
10	CH ₂ CH ₂ CH ₃	CH ₂ CH ₂ CH ₃	4-heptanone
11	CH (CH ₃) ₂	H	2-methyl propionaldehyde
12	CH (CH ₃) ₂	CH ₃	4 methyl butanone
13	CH (CH ₃) ₂	CH ₂ CH ₃	4 methyl 3-butanone
14	CH (CH ₃) ₂	CH ₂ CH ₂ CH ₃	6-methyl 4-hexanone
15	CH (CH ₃) ₂	CH (CH ₃) ₂	2,4 dimethyl 3-pentanone
16	CH ₂ CH (CH ₃) ₂	H	3-methylbuteraldehyde
17	CH ₂ CH (CH ₃) ₂	CH ₃	4 methyl pentanone
18	CH ₂ CH (CH ₃) ₂	CH ₂ CH ₃	5 methyl hexanone
19	CH ₂ CH (CH ₃) ₂	CH ₂ CH ₂ CH ₃	6 methyl heptanone
20	CH ₂ CH (CH ₃) ₂	CH ₂ CH (CH ₃) ₂	1-methyl, 7-methyl 4-heptanone
21	CH ₂ CH ₂ CH ₂ CH ₃	H	valeraldehyde
22	CH ₂ CH ₂ CH ₂ CH ₃	CH ₃	2-hexanone
23	CH ₂ CH ₂ CH ₂ CH ₃	CH ₂ CH ₃	3-heptanone
24	CH ₂ CH ₂ CH ₂ CH ₃	CH ₂ CH ₂ CH ₃	4-octanone
25	CH ₂ CH ₂ CH ₂ CH ₃	CH ₂ CH (CH ₃) CH ₃	2-methyl 4-octanone
26	CH ₂ CH ₂ CH ₂ CH ₃	CH ₂ CH ₂ CH ₂ CH ₃	5-nananone
27	CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	CH ₃	2-heptanone
28	CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	CH ₃	2-octanone

Compound Number	R ₁	R ₂	Starting Material
29	CH (CH ₂ CH ₃)CH ₂ CH ₃	CH ₃	3-ethyl 2-pentanone
30	CH (CH ₃)CH ₂ CH ₃	CH ₃	3-methyl 2-pentanone
31	CH (CH ₃)CH(CH ₃)CH ₂ CH ₃	H	2,3-dimethylpentaldehyde
32		CH ₃	acetophenone
33		H	benzaldehyde
34		CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	1-phenyl 1-heptanone
35		CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	1-phenyl 1-hexanone
36		H	cyclohexanone
37		H	2-phenylacetaldehyde
38		CH ₃	3-phenyl 2-propanone
39		H	3-methyl 3-phenylbutyraldehyde
40		H	3-methyl 3-cyclohexylbutyraldehyde
41		CH ₃	4-methyl 4-phenyl 2-pentanone
42		CH ₃	4-cyclohexyl 4-methyl 2-pentanone
43		H	<i>p</i> -methoxybenzaldehyde
44	CH ₃	CH=C(CH ₃) ₂	1,3-dimethyl-2-butenylidene

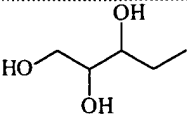
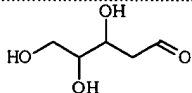
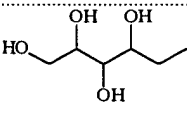
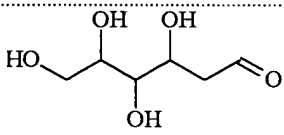

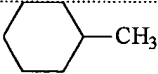

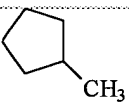

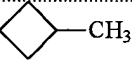
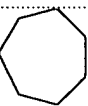
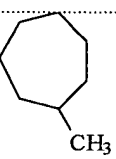
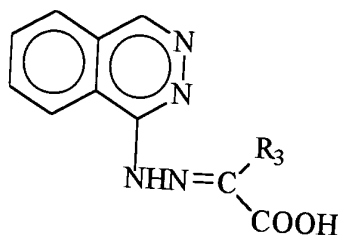
Compound Number	R ₁	R ₂	Starting Material
45	H		 2 -deoxyribose
46	H		 2-deoxyglucose

Table 2

Compound Number	R ₁ and R ₂ together	Starting Material
47		cyclohexanone
48		4-methylcyclohexanone
49		cyclopentanone
50		3-methylcyclopentanone
51		cyclobutanone
52		3-methylcyclobutanone
53		cycloheptanone
54		3-methylcycloheptanone

1 In another embodiment, hydralazine derivatives are prepared by coupling an N-protecting
 2 group to the terminal nitrogen of the highly reactive hydrazino group on hydralazine to
 3 produce a compound having the formula:

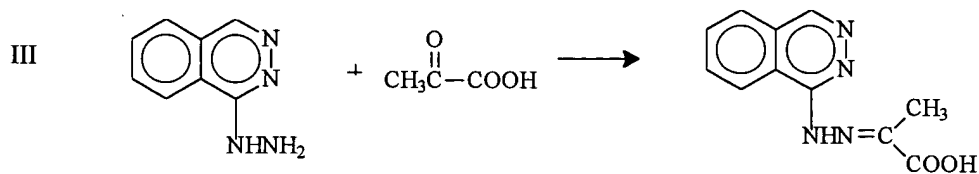


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 6 where R_3 is a branched or straight chain alkyl having from 1 to about 7 carbon atoms,
 7 substituted or unsubstituted aryl, substituted or unsubstituted aralkyl, substituted or
 8 unsubstituted cycloalkyl, substituted or unsubstituted aralkyl, substituted or unsubstituted
 9 alkylcycloalkyl or a group having the formula $(CH_2)_nCOOH$ where n is from 1 to about 7.
 10 In accordance with one aspect of this embodiment of the present invention, R_3 is a
 11 branched or straight chain alkyl having from 1 to about 7 carbon atoms. In accordance
 12 with another aspect of this embodiment of the present invention, R_3 is a substituted or
 13 unsubstituted aryl, substituted or unsubstituted aralkyl, substituted or unsubstituted
 14 cycloalkyl, substituted or unsubstituted aralkyl, substituted or unsubstituted
 15 alkylcycloalkyl. In yet another aspect of this embodiment of the present invention, R_3 is a
 16 group having the formula $(CH_2)_nCOOH$ where n is from 1 to about 7.
 17 Because the hydrazine group readily reacts with the carbonyl group of acids the N-
 18 protected compounds of the present invention can easily be prepared by reacting
 19 hydralazine hydrochloride with acids such as pyruvic acid (III) α -ketoglutarate (IV) as
 20 illustrated generally below:
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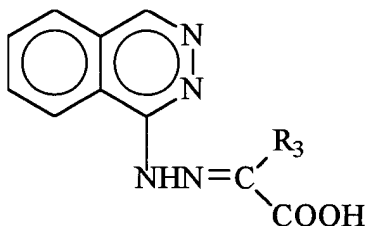
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In accordance with a preferred embodiment of the present invention hydralazine derivatives are prepared by coupling an N-protecting group to the terminal nitrogen of the highly reactive hydrazine group on hydralazine to produce a compound having the formula



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where R_3 a group having the formula $(CH_2)_nCOOH$ where n is from 1 to about 4.

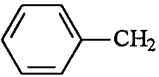

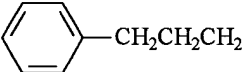
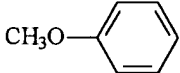
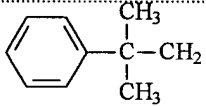

Illustrative compounds in accordance with this embodiment of the present invention are found in Table 3.

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Table 3 - Ketoacids

Compound Number	R ₃	Starting Material
55	CH ₃	Pyruvic acid
56	CH ₂ CH ₃	α-ketobutyric acid
57	CH ₂ CH ₂ CH ₃	α-ketopentanoic acid
58	CH (CH ₃) ₂	α-ketoisovaleric acid
59	CH ₂ CH (CH ₃) ₂	α-ketoisocaproic acid
60	CH ₂ CH(CH ₃)CH ₂ CH ₃	3-ethyl, 3 methyl pyruvic acid
61	CH ₂ CH ₂ CH ₂ CH ₃	α-ketohexanoic acid
62	CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	α-ketoheptanoic acid
63	CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₃	α-ketooctanoic acid
64		α-ketophenylpyruvic acid
65		α-ketophenylglyoxylic acid
66		α-keto 4-phenylbutyric acid
67		α-keto <i>p</i> -methoxyphenyl glyoxylic acid
68		4-methyl, 4-phenyl α-ketopentanoic acid
69		α-ketocyclohexylglyoxylic acid

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Compound Number	R ₃	Starting Material
70	CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ COOH	α-ketodecanedioic acid
71	CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ COOH	α-ketononanedioic acid
72	CH ₂ CH ₂ CH ₂ CH ₂ CH ₂ COOH	α-ketooctanoic acid
73	CH ₂ CH ₂ CH ₂ CH ₂ COOH	α-keto heptanoic acid
74	CH ₂ CH ₂ CH ₂ COOH	α-keto hexanoic acid
75	CH ₂ CH ₂ COOH	α-ketoglutaric acid
77	CH ₂ COOH	α-keto butanedioic acid
78	COOH	α-keto glyoxalic acid

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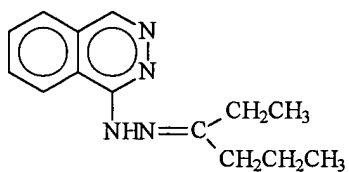
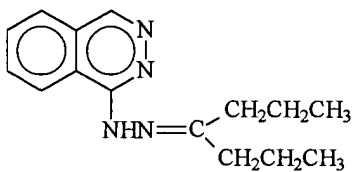
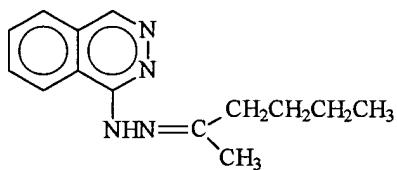
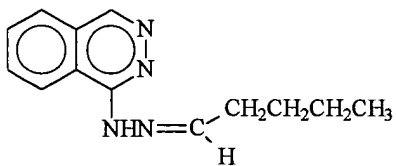
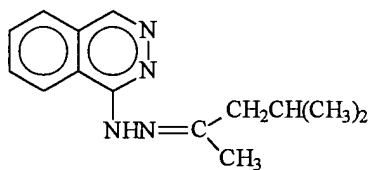
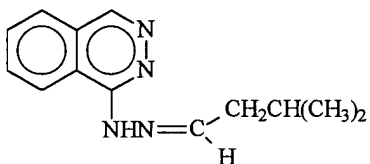
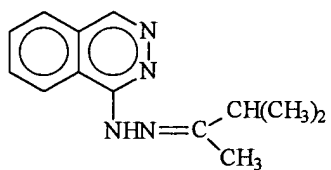
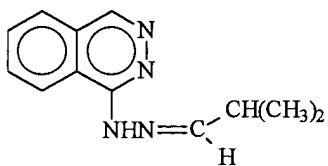
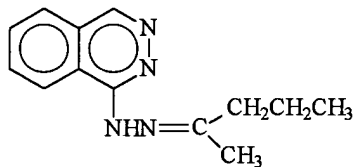
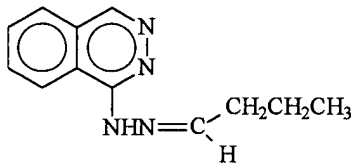
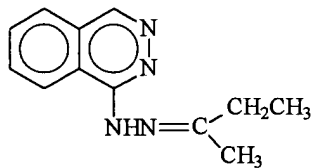
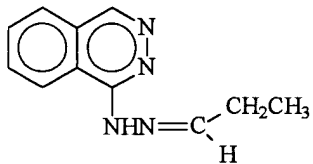
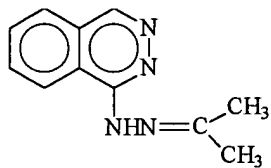
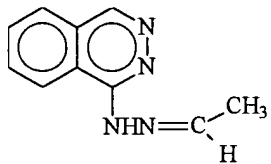
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1 Illustrative compounds in accordance with the present invention include the following:

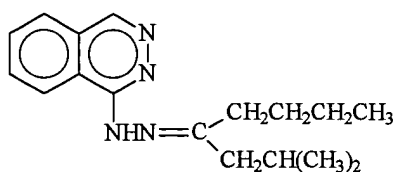
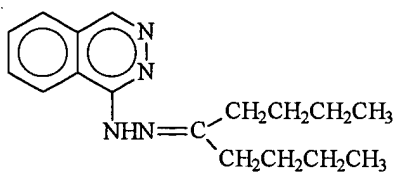
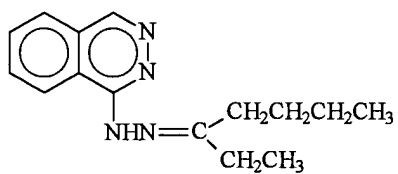
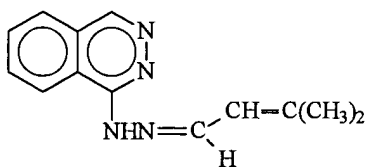
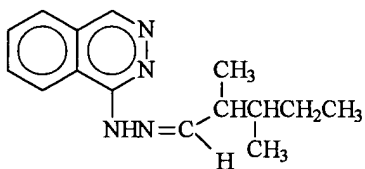
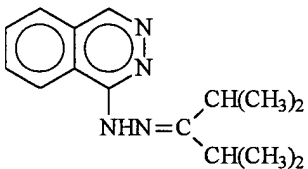
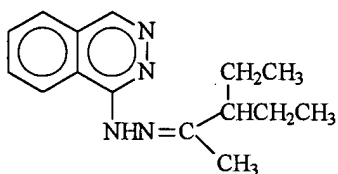
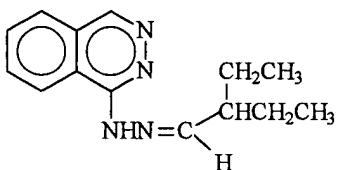
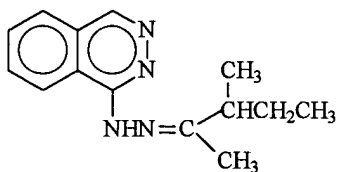
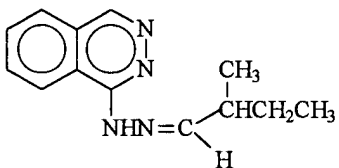
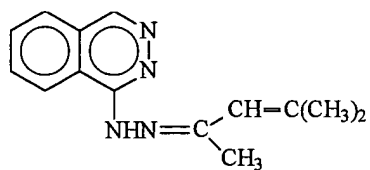
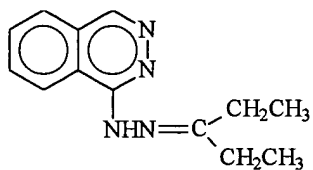
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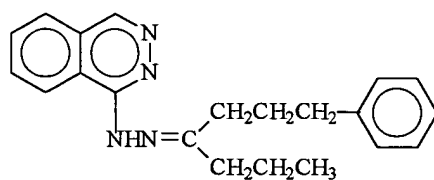
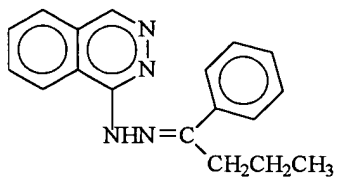
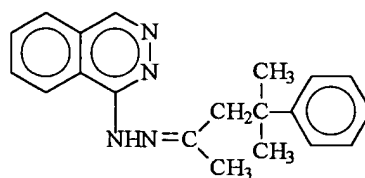
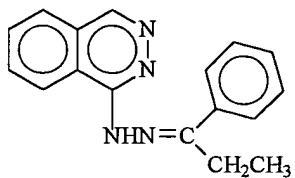
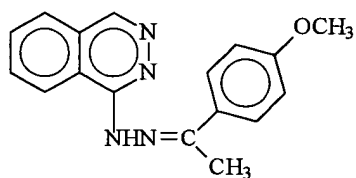
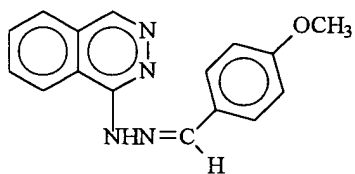
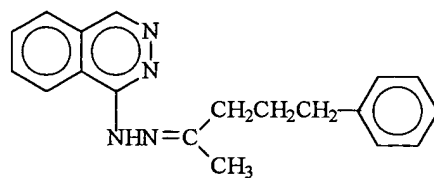
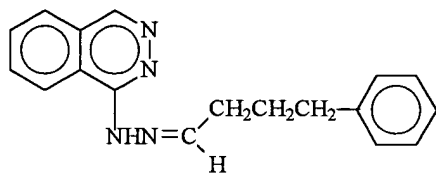
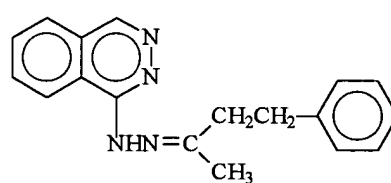
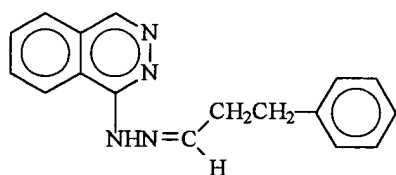
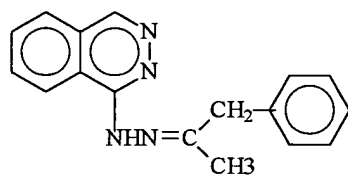
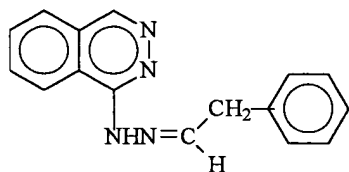
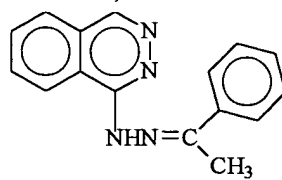
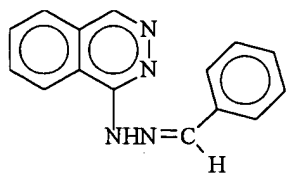
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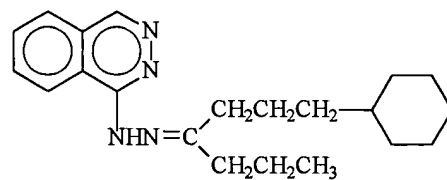
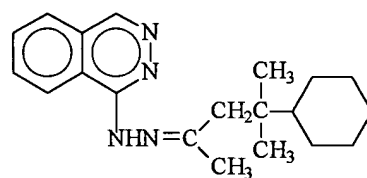
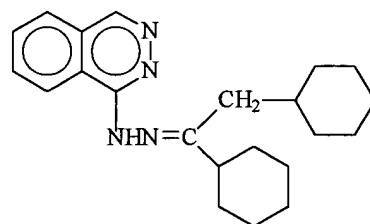
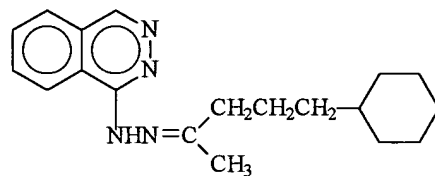
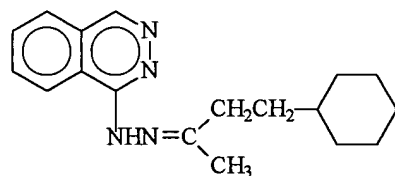
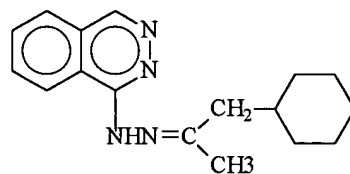
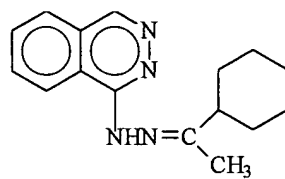


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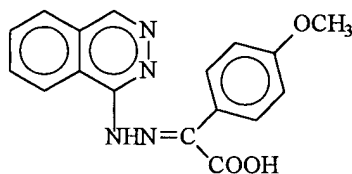
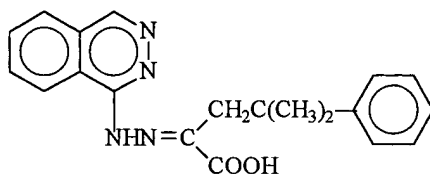
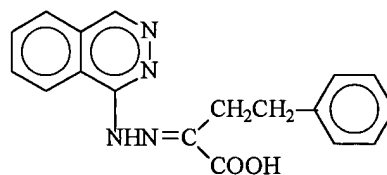
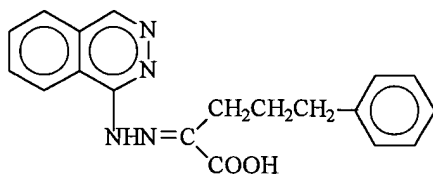
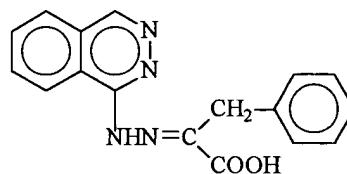
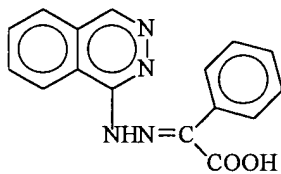
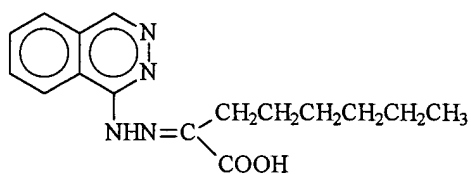
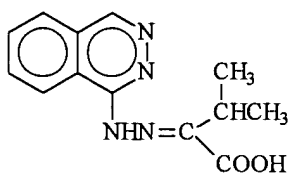
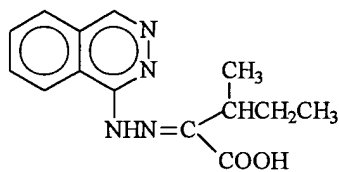
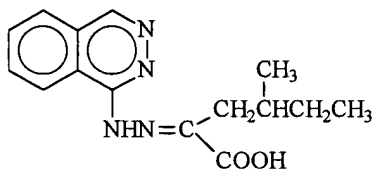
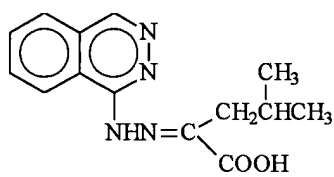
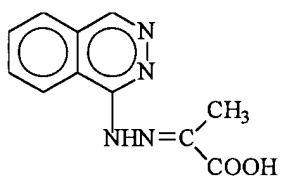


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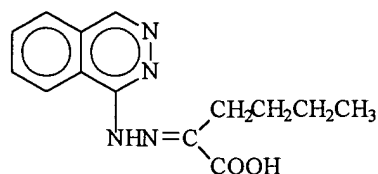
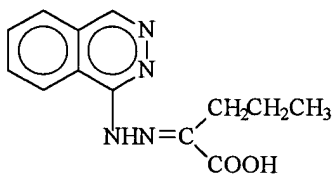
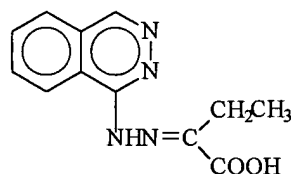
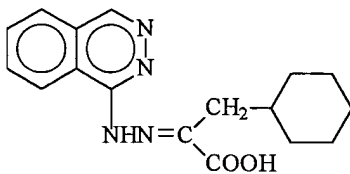
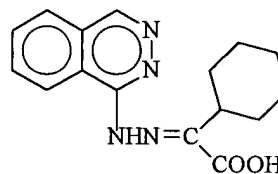
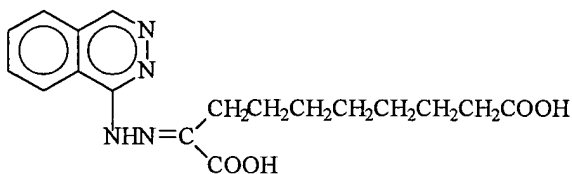
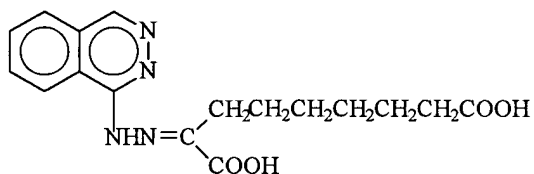
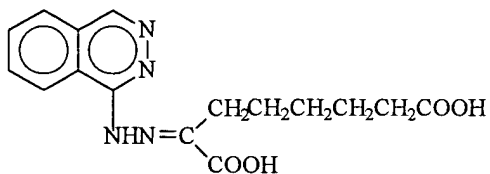
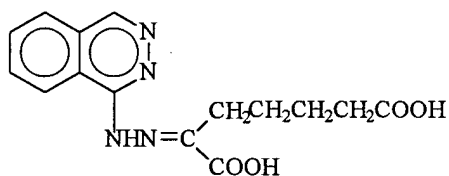
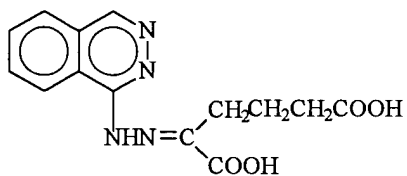
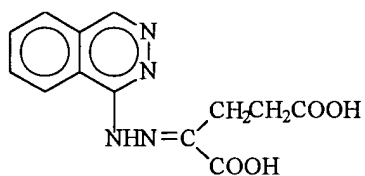
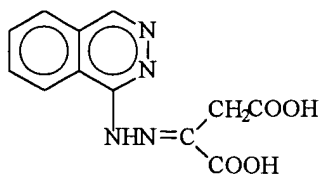




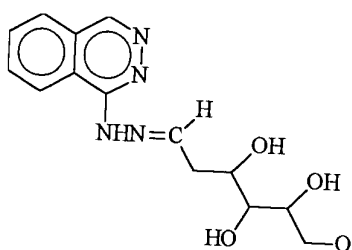
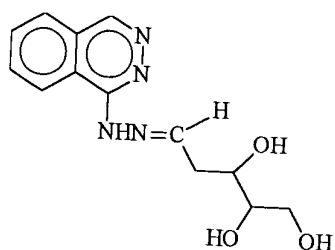
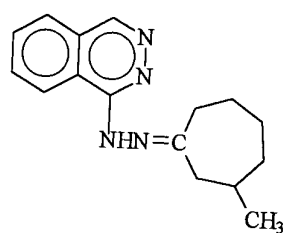
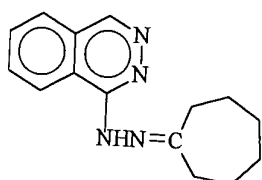
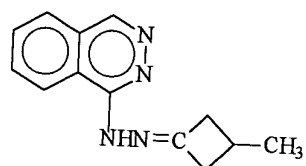
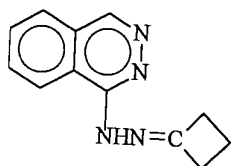
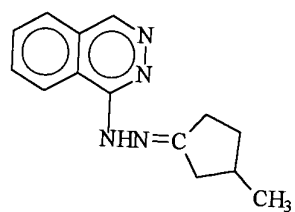
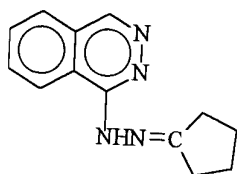
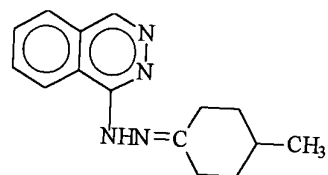
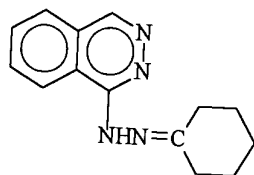
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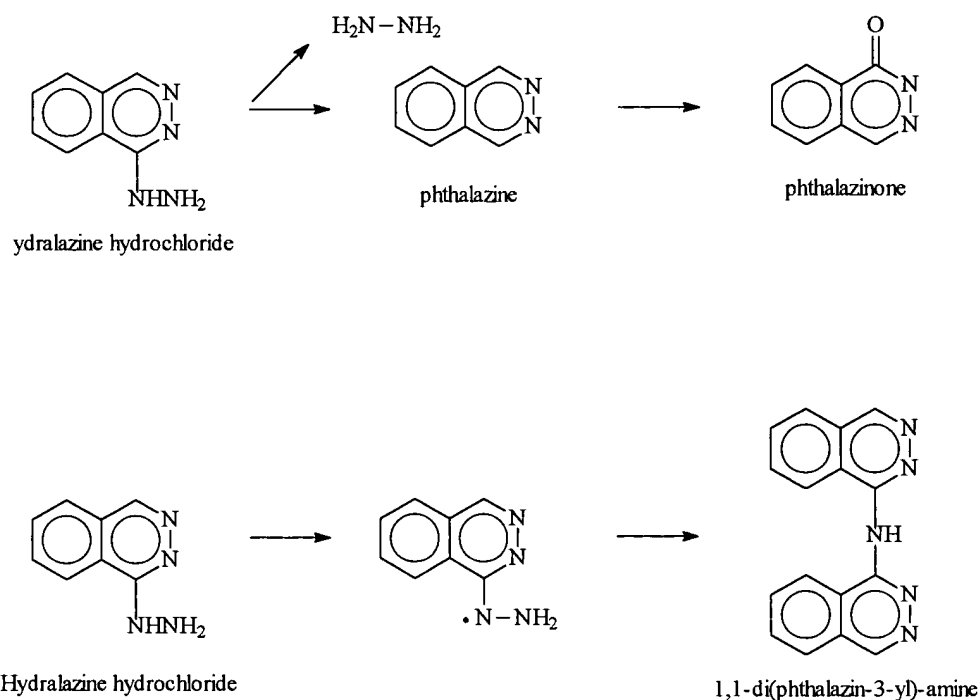


1



1 In accordance with the present invention, stability with respect to the hydralazine
 2 compositions refers to both the chemical and physical integrity of the composition.
 3 Hydralazine hydrochloride, with a pK of about 7.3 is most stable at a pH of about 3.4 to
 4 about 4.4, and is unstable at high pH where it degrades into phthalazine, phthalazinone
 5 and hydrazine. Because injectable hydralazine pharmaceutical solutions are currently
 6 required to contain less than 10 ppm of hydrazine, sterile injectable hydralazine solutions
 7 have a pH of between 3.4 and 4.4. Even stored at a pH of about 3.4, sterile injectable
 8 hydralazine solutions produce detectable amounts of phthalazine, phthalazinone and
 9 hydrazine over time during storage. These sterile injectable hydralazine solutions are
 10 further believed to undergo degradation to insoluble polymeric products through the
 11 highly reactive hydrazino group as illustrated below:

Degradation of Hydralazine on Storage



12

1 Although confirmation is not yet available, it is believed that formation of the submicron
2 particles in injectable hydralazine solutions results from the insoluble polymers of
3 hydralazine generated through degradation during storage. In accordance with the present
4 invention, the stability of hydralazine compositions during storage is determined by
5 monitoring the degradation products phthalazine, phthalazinone and hydrazine as well as
6 the generation of insoluble polymeric products. Even stored at a pH of 3.4, sterile
7 injectable hydralazine solutions produce detectable amounts of submicron particles over
8 time during storage. In accordance with a most preferred embodiment of the present
9 invention, the stability of the pharmaceutical compositions containing hydralazine is
10 improved such that the shelf life of pharmaceutical compositions containing hydralazine
11 is significantly extended beyond 12 months storage and preferably beyond 24 months
12 storage.

13
14 It is an object of the present invention that the stability of the N-protected hydralazine
15 compounds is improved compared to that of unconjugated hydralazine. The stability of
16 the N-protected hydralazine compounds of the present invention varies depending upon
17 the nature of the protecting group; however, many of the compounds in accordance with
18 the present invention are acid-labile. Consequently, these compounds are stored at higher
19 pH. In contrast to the sterile injectable hydralazine solutions which are stored at a pH of
20 about 3.4 to 4.4, the hydralazine containing pharmaceutical compositions in accordance
21 with the present invention are preferable stored at a pH greater than 4.4. In a more
22 preferred embodiment of the present invention, the hydralazine containing
23 pharmaceutical compositions of the present invention are stored at a pH of about 5 to
24 about 8. The pH stability of the compounds of the present invention is easily measured by
25 testing solutions up to 24 months using a HPLC method having a lower limit of
26 sensitivity of 0.0125 uM.

27
28 In a preferred embodiment of the present invention, stable hydralazine solutions contain
29 less than about 10 ppm (parts per million) and more preferable less than about 3 ppm
30 hydrazine. In a more preferred embodiment of the present invention, stable hydralazine

1 compositions contain less than about 1% by weight of degradation products including
2 phthalazine and phthalazinone. In a more preferred embodiment of the present invention,
3 stable hydrazine solutions are essentially particle-free. That is, the presence of particulate
4 matter or particles in injectable hydralazine compositions is not detectable by inspecting
5 the hydralazine solutions in both an upright and inverted position. In a most preferred
6 embodiment of the present invention, the formation of submicron particles in liquid
7 pharmaceutical compositions containing hydralazine during storage is significantly
8 reduced and particles are not detectable from about 18 to about 24 months after
9 completion of manufacturing and storage was initiated. In one embodiment of the present
10 invention, injectable hydralazine formulations do not form small yellow-green particles
11 from 1 to about 2 months after storage when hydralazine is stored at 40° C and after from
12 6 to about 9 months storage at 25° C.

13
14 In accordance with the present invention, particulate matter consists of mobile randomly
15 sourced extraneous substances, other than gas bubbles, that cannot be quantitated by
16 chemical analysis due to the small amount of material that it represents and to its
17 heterogeneous composition. Particulate matter (particles) in the injectable solutions for
18 parenteral use in accordance with the present invention is determined based on visual
19 inspection and by measured light obscuration procedures. Particles having a diameter of
20 about 50 microns can be measured by visual inspection. The light obscuration procedures
21 are performed for the purpose of enumerating subvisible extraneous particles having sizes
22 than about 50 microns. In accordance with the present invention, detection of particulate
23 matter by light obscuration is preferably performed with a suitable electronic, liquid-
24 borne particle counting system that uses a light-obscuration sensor with a suitable
25 sample-feeding device. A variety of suitable devices of this type are commercially
26 available.

27
28 In accordance with the one embodiment of the present invention, an injectable
29 pharmaceutical composition is considered stable if the average number of particles of
30 about 10 microns in the composition does not exceed 6,000. In accordance with another

embodiment of the present invention, an injectable pharmaceutical composition is considered stable if the average number of particles of about 25 microns in the composition does not exceed 600. In accordance with yet another embodiment of the present invention, a pharmaceutical composition is considered stable if no particles are visible. In accordance with a preferred embodiment of the present invention, an injectable hydralazine containing pharmaceutical composition is particle-free if the average number of particles of about 10 microns in the composition does not exceed 6,000, the average number of particles of about 25 microns in the composition does not exceed 600, and no particles are visible.

Acid labile derivatives of hydralazine have been reported by a number of researchers in an effort to identify and characterize the metabolites of hydralazine including Clementi et al. in Journal of Pharmacological and Experimental Therapeutics 222(1): 159- 165 (1982) found that certain acid-labile hydralazine derivatives were also plasma labile and are pharmacologically active and are endogenously hydrolyzed to parent hydralazine after intravenous administration. Clementi et al. report that, although differences in the pharmacological properties between the labile derivatives related to the time course of parent hydralazine generation in plasma exist, the hydrolysis of the labile derivatives may be nearly complete. Although differences in the extent and rate of appearance of hydralazine in plasma are reported by Clementi et al., the extent and rate of appearance is therapeutically similar to that of hydralazine after administration of hydralazine. Differences between the stability of hydralazine derivatives in plasma in vitro and the same compounds in vivo suggest that plasma-labile derivatives of hydralazine might be altered in the tissues as well as in the plasma.

The N-protected compounds produced from reaction with ketones, aldehydes or ketoacids in accordance with the present invention are used in the preparation of a pharmaceutical dosage form intended for human use. In the case of manufacturing a sterile injectable dosage form suitable for intravenous administration to a patient, the N-protected compounds are dissolved in an appropriate solution for parenteral

1 administration and filled into bottles, vials, syringes or ampules with a pharmaceutically
2 acceptable diluent under sterile manufacturing conditions. Upon completion of
3 manufacturing this sterile injectable solution, the filled bottles, vials, syringes or ampules
4 are stored under appropriate storage conditions. In the case of an oral dosage form, the N-
5 protected compounds are mixed with pharmaceutically acceptable fillers and excipients
6 in a syrup, capsule or tablet.

7

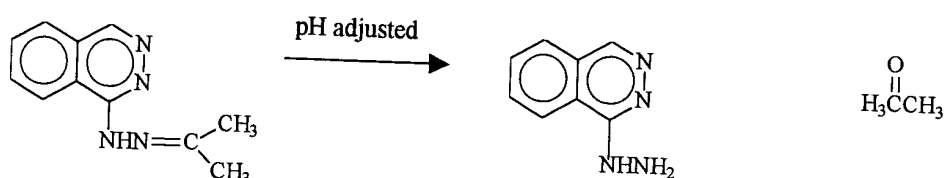
8 In one embodiment of the present invention, the N-protected compounds are formulated
9 in a concentrated sterile solution for dilution at a concentration of from about 10 to about
10 30 mg/ml (by weight), and preferably at a concentration of about 20 mg/ml. Most
11 preferably, these compounds are formulated in sterile water for injection at a
12 concentration of 20 mg/ml. In accordance with this embodiment of the present invention,
13 the pH of the injection solution during storage is from about 7.4 to about 9.0, and
14 preferably from about 8.0 to 8.5.

15

16 In another one embodiment of the present invention, the N-protected compounds are
17 formulated in a more dilute concentration in a sterile solution. In accordance with a
18 preferred embodiment of the present invention, these compounds are formulated at a
19 concentration of from about 0.5 to about 10 mg/ml (by weight), and preferably at a
20 concentration of about 5 mg/ml. Most preferably, these compounds are formulated in
21 sterile water for injection at a concentration of 5 mg/ml.

22

23 After storage of the sterile injectable dosage form containing the N-protected compounds
24 of the present invention, the N-protecting group is removed from the parent hydralazine
25 molecule immediately prior to injection into a patient. In one embodiment of the present
26 invention, the pH of the sterile injectable solution containing the acid-labile compound is
27 adjusted so that N-protecting group is released from hydralazine and the N-protecting
28 group remains in the injection solution. This is illustrated by the following reaction
29 scheme:



1

2 By way of example, a vial containing 2 ml of the N-protected compounds of the present
 3 invention formulated in a concentrated sterile solution for dilution at a concentration of
 4 about 20 mg/ml at pH 8.0, is diluted prior to use with a sufficient volume of sterile water
 5 for injection having a stabilizing effective pH. Preferably, the pH of the diluted solution
 6 is from about 3.0 to about 6.0.

7

8 Alternatively, the acid-labile compounds of the present invention are formulated into an
 9 oral dosage form such as a syrup, capsule or tablet. In this case, the syrup, capsules or
 10 tablets containing the N-protecting compounds can be dosed to a patient without prior
 11 manipulation of the pH. In this case, as the oral dosage form reaches the highly acidic
 12 conditions of the stomach and the N-protecting group is removed from the parent
 13 hydralazine molecule prior to absorption. As in another embodiment of the present
 14 invention, the N-protecting group is released from hydralazine; however, the N-
 15 protecting group will be absorbed and metabolized as it travels through the
 16 gastrointestinal system.

17

18 In the case of plasma-labile compounds of the present invention, there is no need to
 19 adjust the pH of the composition so that N-protecting group is removed from the parent
 20 hydralazine compound. In this case, the compounds of the present invention are
 21 administered to the patient and the N-protecting group is removed in the plasma after
 22 administration such that the extent and rate of appearance of hydralazine in the plasma is
 23 therapeutically similar to that of hydralazine after administration. In accordance with the
 24 present invention, the plasma-labile compounds are therapeutically similar to hydralazine
 25 with regard to vasopressor activity. It is contemplated that some differences in the extent
 26 and rate of appearance of hydralazine in the plasma between these plasma-labile

1 compounds and hydralazine will occur. Nevertheless, their extent and rates of appearance
2 in patients are to be considered therapeutically similar if these differences are medically
3 insignificant or these compounds have the same clinical effect when administered to
4 patients when administered under similar clinical conditions.

5

6 It is an object of the present invention to stabilize pharmaceutical compositions during
7 manufacturing so that the hydralazine does not react with metal components of the
8 manufacturing system. It is a further object of the present invention to stabilize injectable
9 pharmaceutical compositions containing hydralazine and reduce the discoloration of the
10 hydralazine solution when diluted with conventional pharmaceutical diluents containing
11 trace amounts of metals such as of Cu^{+2} , Fe^{+2} and Fe^{+3} . Accordingly, the N-protected
12 compounds of the present invention have a reduced capacity to complex with or
13 otherwise react with metals in the manufacturing solutions, storage solutions and diluent
14 solutions employed in the hospital. In accordance with the present invention, the
15 pharmaceutical compositions are essentially metal free and contain essentially metal-free
16 hydralazine. The presence of metals complexed with hydralazine, as measured by the
17 presence of color or reactivity with spin-label probes, is a clear indication that the
18 pharmaceutical compositions are not metal free and the compositions do not contain
19 metal-free hydralazine.

20

Example 1**Preparation of 1-hydrazinophthalazine**

1-chlorophthalazine (30 parts) is heated for two hours in a mixture of 100 parts by volume of ethyl alcohol and 90 parts by volume of hydrazine hydrate. After filtering, 1-hydrazinophthalazine crystallizes in yellow needles on cooling. The yellow needles are filtered with suction and washed with cold ethyl alcohol, and recrystallized from methyl alcohol (mp 172-178° C). On warming in alcohol or aqueous hydrochloric acid, the hydrochloride is obtained (mp 273° C).

Example 2**Preparation of 1-hydrazinophthalazine α -ketoglutarate hydrazone**

1-hydrazinophthalazine hydrochloride (395 mg; 2 mmol) is dissolved in 5 ml of water. To this an aqueous solution of α -ketoglutaric acid (1 g; 7 mmol) is added and the reaction mixture is allowed to stand overnight at room temperature. The solid precipitate is filtered off and dried in *vacuo* to yield 510 mg of 1-hydrazinophthalazine α -ketoglutarate hydrazone (88% theoretical yield). [British Journal of Clinical Pharmacology 5: 489-494 (1978)]

Example 3**Preparation of 1-hydrazinophthalazine formaldehyde hydrazone**

Formaldehyde (0.3 g, 6.82 moles) is added with stirring to 500 ml of 0.05 M phosphate buffer at pH 7.4 and 0.5 g (2.54 mmoles) of 1-hydrazinophthalazine at 37° C. The reaction mixture is stirred at 37° C for 10 min and then filtered. The filtrate is dried in *vacuo* to yield a solid residue. Recrystallization from chloroform-ether gives 0.440 g

1 (92%) of the 1-hydrazinophthalazine formaldehyde hydrazone as off-white crystals, mp
2 108-110° C. [Journal of Pharmacological Sciences 68(12):1524-1526 (1979)]

3 **Example 4**

5 **Preparation of 1-hydrazinophthalazine acetaldehyde hydrazone**

6 Acetaldehyde (0.3 g, 6.82 moles) is added with stirring to 500 ml of 0.05 M phosphate
7 buffer at pH 7.4 and 0.5 g (2.54 mmoles) of 1-hydrazinophthalazine at 37° C. The
8 reaction mixture is stirred at 37° C for 10 min and then filtered. The filtrate is dried *in*
9 *vacuo* to yield a solid residue. Recrystallization from chloroform-ether gives 0.440 g
10 (92%) of the 1-hydrazinophthalazine acetaldehyde hydrazone as off-white crystals, mp
11 108-110° C. [Journal of Pharmacological Sciences 68(12):1524-1526 (1979)]

13 **Example 5**

15 **Preparation of 1-hydrazinophthalazine 2-butanone hydrazone**

16 A 10 parts 1-hydrazinophthalazine HCL (0.05 Molar) in 50% methanol-water are mixed
17 with 1 part 2-butanone. After evaporation of solvents, 1-hydrazinophthalazine 2-butanone
18 hydrazone is crystallized from ethanol-heptane (78% yield). [The Journal of
19 Pharmacology and Experimental Therapeutics 205 (2): 418-425 (1978)].

21 **Example 6**

23 **Preparation of 1-hydrazinophthalazine acetone hydrazone**

24 1-hydrazinophthalazine HCL (395 mg; 2 mmoles) is dissolved in 2.5 ml of acetone and
25 allowed to react for 1 hour. The solvent is evaporated and the slightly yellow material is
26 dried *in vacuo* to yield 411 mg of 1-hydrazinophthalazine acetone hydrazone. (99% of
27 theoretical yield). [British Journal of Pharmacology 61: 345-349 (1977)]

Example 7**Preparation of 1-hydrazinophthalazine pyruvate hydrazone (a)**

1-hydrazinophthalazine HCL (5 g; 25 mmoles) is dissolved in 50 ml 0.1 M sodium phosphate buffer pH 7.4, and a solution of sodium pyruvate (11 g; 100 mmoles) in 30 ml of 0.1 M sodium phosphate buffer pH 7.4 is added while stirring vigorously. The solution becomes distinctively yellow almost immediately and the hydrazone is precipitated slowly. After standing overnight at 4° C, the yellow crystalline product is filtered off and washed with cold distilled water. The residue is recrystallized from hot ethanol-water to yield 4.3 g of 1-hydrazinophthalazine pyruvate hydrazone (70% of theoretical yield). [Journal of Chromatography 187:171-179 (1980)]

Example 8**Preparation of 1-hydrazinophthalazine pyruvate hydrazone (b)**

1-hydrazinophthalazine HCL (1g) is mixed with acetone (0.38 g) in 30 ml water at room temperature and the solution is magnetically stirred for 10 minutes. Precipitated 1-hydrazinophthalazine acetone hydrazone is collected by filtration as yellow crystals and dried over CaCl₂ [Journal of Pharmaceutical Sciences 85(3): 326-329 (1996)]

Example 9**Preparation of 1-hydrazinophthalazine anisaldehyde hydrazone**

1-hydrazinophthalazine HCL (395 mg; 2 mmoles) is dissolved in 2.5 ml of anisaldehyde and allowed to react for 1 hour. The solvent is evaporated and the slightly yellow material is dried *in vacuo* to yield 411 mg of 1-hydrazinophthalazine anisaldehyde hydrazone. [Journal of Chromatography 126: 527-534 (1976)]

Example 10**Single dose protocol in SHR [spontaneously hypertensive rats]**

Adult male spontaneously hypertensive rats (SHR) weighing between 250 and 350 g are maintained on standard rat chow and water *ad libitum*, and are prepared for cardiovascular studies by implantation of chronic aortic and jugular polyethylene catheters. Four days are allowed for recuperation from surgery. Blood pressure is measured and recorded by a Grass model 7D polygraph (Grass Instrument Co., Quincy, MA) through individual Statham P23Gb pressure transducers. An estimate of mean arterial pressure (MAP) is obtained by maximal electronic dampening of the input signal. During blood pressure measurements the animals are placed in Plexiglas cages with minimal restraint. The MAP should range from 150 to 170 mm Hg.

Each rat receives at least three different doses of i. v. bolus injections of hydralazine and the compounds of Examples 2 through 8 dissolved in a solution of sterile water for injection at pH 3.4. Drug administrations are separated by 4 days to permit complete dissipation of drug effect. The volume of each injection is 0.1 ml/100 g by weight for doses of 2.5 through 12.5 $\mu\text{mol/kg}$. At doses of 20 $\mu\text{mol/kg}$ or greater, the volume of each injection is increased to 0.2 ml/kg to permit complete solubilization of the N-protected hydralazine compounds. Hydralazine is dissolved in 30% EtOH-0.9% NaCl. After drug administration, vasopressor activity is determined by measuring the mean arterial pressure (MAP) at 2, 4, 6, 8, 10, 15 and 30 min and then every 30 min for 180 min. Dose response results are assessed by comparing the average peak change in MAP after administration at each dose of compound administered.

Example 11**Stability of hydralazine hydrazones in storage solutions**

The stability of the N-protected hydralazine compounds of the present invention is tested in solutions having a pH of 3.0, 4.0, 5.0, 6.0 7.0, 8.0, 9.0 and 10.0 using a HPLC method

1 having a lower limit of sensitivity of 0.0125 uM. The amounts of hydrazine, hydralazine
2 and the N-protected hydralazine compounds in an injectable formulation are measured
3 using HPLC immediately after storing the solutions (T_0), 1 week, 2 weeks, 1 month, 2
4 months, 3 months, 6 months, 9 months, 18 months and 24 months after storage at 25° and
5 60° C. In addition, the stored solutions are visually inspected in both the upright and
6 inverted positions for the presence of particulate matter immediately after storing the
7 solutions (T_0), 1 week, 2 weeks, 1 month, 2 months, 3 months, 6 months, 9 months, 18
8 months and 24 months after storage at 25° and 60° C. The amounts of hydrazine,
9 hydralazine and the N-protected hydralazine compounds in an injectable formulation are
10 also measured using HPLC immediately after storing the solutions (T_0), 1 week, 2 weeks,
11 1 month, 2 months, 4 months, and 6 months after storage at 40° C. In addition, the stored
12 solutions are visually inspected in both the upright and inverted positions for the presence
13 of particulate matter immediately after storing the solutions (T_0), 1 week, 2 weeks, 1
14 month, 2 months, 4 months, and 6 months after storage at 40° C. The presence of
15 particulate matter or small yellow-green particles over time is a measure of stability.
16

17 Example 12

18 **Lability of hydralazine hydrazones in administration solutions**

19 The lability of the N-protected hydralazine compounds of the present invention is tested
20 in solutions having a pH of 3.0, 4.0, 5.0, 6.0 7.0, 8.0, 9.0 and 10.0 using a HPLC method
21 having a lower limit of sensitivity of 0.0125 uM. After adjusting the pH of the storage
22 solution containing the N-protected hydralazine compounds, the hydrazones and
23 hydralazine are measured at 10,20,30,60 and 120 minutes.
24

25
26 The present invention has been described in detail using specific examples to illustrate
27 the preferred embodiments of the invention; however, it will be obvious to those skilled
28 in the art that various modifications thereto can be made without departing from the spirit
29 and scope thereof.